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Predicting 2,4-Dichlorophenoxyacetic Acid Ester Transformation Rates in Periphyton-Dominated Ecosystems

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16. ABSTRACT

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Predicting 2,4-Dichlorophenoxyacetic Acid Ester Transformation Rates in Periphyton-Dominated Ecosystems

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Using batch cultures, we determined transformation rate coefficients for microbial transformation of 2,4-dichlorophenoxyacetic acid butoxyethyl ester (2,4-DBE) in periphyton-dominated ecosystems. Rates of 2,4-DBE loss were measured over short periods of time (usually less than 10 h), and first-order transformation rate coefficients (k_1) were determined under the specific conditions of low 2,4-DBE concentrations and no growth. Values for k_1 were divided by total plate counts and by biomass measured as ash-free dry weight to give second-order rate coefficients (k_b and k_{AFDW}, respectively) for use in predictive models. Using periphyton attached to Teflon strips, we also determined second-order rate coefficients based on the ratio of colonized surface area to container volume (k_A). Mean second-order rate coefficients were used to predict 2,4-DBE transformation rates in microcosms having diverse chemical and biological environments. The observed transformation rates among the microcosms were most accurately predicted by using k_A.

Our approach to predicting the microbial transformation rate of xenobiotic chemicals in aquatic ecosystems involved using batch cultures of microbiota to determine microbial transformation rate coefficients. The approach also involved testing the applicability of the rate coefficients to predicting transformation rates under controlled conditions in large laboratory ecosystems. Using Michaelis-Menton kinetics (4), we determined first-order transformation rate coefficients (k_1) for the loss of xenobiotic chemicals in batch cultures with specific organism and substrate concentrations. The conditions were (i) an organism concentration sufficiently high that no measurable growth (increase in enzyme concentration) occurred during the experiments, and (ii) a very low xenobiotic chemical concentration, so that the substrate concentration was below K_m, the half-saturation concentration of the enzyme system. To ensure no significant organism growth during the experiments, 10^7 to 10^9 CFU of bacteria per liter were used, and transformation rates were measured during short incubation periods (usually less than 10 h). Nonsaturation of the enzyme system was indicated by transformation rates being first-order relative to the substrate concentration over at least a one-order-of-magnitude range in substrate concentration. We expected that the transformation rate coefficients determined under these conditions would be applicable to the transformation rates of chemicals for commonly found environmental concentrations at or below the microgram-per-liter range. These coefficients would not be applicable to spill or discharge situations, for which transformation rates above K_m are zero-order relative to substrate concentration and first-order relative to biomass (enzyme concentration). The first-order rate coefficient can be normalized to an environmental parameter, such as respiration, primary productivity, microbial biomass, the transformation rate coefficient of an indicator substrate, or any other parameter that correlates with the transformation rate of the xenobiotic chemical, to give a second-order rate coefficient.

We did not expect any single rate coefficient to be satisfactory for predicting microbial transformation rates for all chemicals in all environmental situations. Our goal was to develop suitable approaches to determining rate coefficients that could be easily used by investigators at the Environmental Protection Agency and in industry to develop data on the microbial transformation of environmental pollutants. Preferably, coefficients could be determined with equipment or techniques available at most laboratories, and the coefficients would not be site specific, i.e., would not require the actual introduction of a chemical to the site of interest to determine transformation rates at the site or in cultures taken from it.

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In this study, we determined second-order transformation rate coefficients for the microbial transformation of a herbicide, 2,4-dichlorophenoxyacetic acid butoxyethyl ester (2,4-DBE). The second-order transformation rate coefficients that we evaluated with 2,4-DBE included those based on (i) total plate counts (k_b), (ii) biomass measured as ash-free dry weight (AFDW) (k_{AFDW}), and (iii) the ratio of colonized surface area to container volume (k_A).

We tested the accuracy of predictions made with k_b, k_{AFDW}, and k_A for transformation rates of 2,4-DBE in a series of 250-liter, continuous-flow microcosms in a large controlled-environment chamber (2). Mean values were used because our predictive model was designed to incorporate non-site-specific coefficients. The large microcosms were used not to simulate any particular field site, but to test the applicability of second-order rate coefficients under the wide range of parameter values on which the coefficients were based. For example, predictions of transformation rates made by using k_b were evaluated with a series of large microcosms having a wide range of total bacterial concentrations. Because the large microcosms were not designed to vary the ratio of surface area to volume, k_A was also tested by using a series of beakers stirred with paddles in which the surface area/volume ratios were varied.

MATERIALS AND METHODS

Organisms. Grab samples of aufwuchs (periphyton plus free-floating mats) were collected from four freshwater field sites near Athens, Ga. These sites included two shallow shoals areas, Harris Shoals and Watson Mill, and two rivers, the Apalachee and the Oconee. Periphyton was collected by colonizing Teflon strips in laboratory ecosystems and in channels at a municipal sewage treatment facility just before chlorination treatment.

The colonies were grown long enough to ensure fully mature transformation capacity. 2,4-DBE transformation rates were maximum for strips colonized with periphyton for at least 1 week in the sewage treatment plant or 5 weeks in laboratory microcosms containing much lower organic nutrient concentrations. These times were therefore used as the minimum colonization times for periphyton in our studies.

Axenic cultures of fungi and algae in dilute nutrient broth were spiked with 2,4-DBE to a concentration of approximately 50 μg/liter. The cultures were incubated in a shaker incubator at 20°C for 24 h and then tested for the loss of 2,4-DBE.

Biomass determinations measured as AFDW and total plate counts were done by procedures reported earlier (2). The conditions used for total plate counts were not designed to give maximum counts for each site sampled. The method was chosen to index transformation rates to a water quality parameter commonly measured for field data.

Gas-liquid chromatography. 2,4-DBE concentrations in the samples were determined by gas-liquid chromatography. Chromatography conditions were the same as in earlier studies (2), and the extraction efficiency was 101% (n = 54; standard deviation, 6%).

Kinetic studies. 2,4-DBE transformation rates were investigated by using batch cultures of either blended aufwuchs or intact periphyton-colonized Teflon strips. All rates were determined for microbiota in the water from which the samples were taken. For each aufwuchs batch culture, k_1 values were determined for grab samples in a 250-ml Erlenmeyer flask. Aufwuchs samples were collected in triplicate, and some field sites and microcosms were sampled for aufwuchs on more than one date. However, as a quality control for the data, the CFU, k_1, and k_b values were reported for each flask culture only when the standard error of regression for 2,4-DBE data was less than 15% and the CFU count had a coefficient of variation among triplicate plates of less than 30% (see Table 1). Flasks were spiked to an initial 2,4-DBE concentration of 50 μg/liter and shaken in a fluorescent-lighted incubator at 20°C. For plating the cultures before spiking, aufwuchs samples were blended for 5 s in an electric blender. To obtain intact periphyton, colonized Teflon strips were fastened into 2-liter beakers and stirred with stainless steel paddles driven by an electric motor. The beakers were spiked to an initial 2,4-DBE concentration of 150 μg/liter and incubated at ambient laboratory temperature. Four or more strips of various sizes gave a range of surface area-to-volume ratios from 0.0024 to 0.0175 m^2/liter. To approximate site turbulence in our large laboratory microcosms, we adjusted the rotation rate of beaker paddles to give an approximate Reynolds number of 6,000. Before constructing the paddles, we did one experiment designed to test the effects of turbulence on k_1 with propellers. The water in the periphyton beaker cultures was autoclaved. All cultures were at approximately neutral pH or lower; therefore no measurable 2,4-DBE chemical hydrolysis occurred.

Triplicate plate counts were determined for each batch culture at the beginning or end of the rate experiments. Rate experiments were usually conducted for less than 10 h, so a constant microbial concentration was assumed. Microbial transformation was rapid; a minimum of two half-lives transpired during an average time of 10 h. Normally 10 samples were taken to determine the rate of 2,4-DBE loss, but when transformation was very rapid, as few as 5 samples were taken. The loss of 2,4-DBE was a first-order process with respect to the 2,4-DBE concentration, and no lag in the disappearance rate occurred in any batch culture. k_1 was calculated by least-squares regression of the logarithms of 2,4-DBE concentration in batch cultures over time. As determined by high-pressure liquid chromatographic analysis, 2,4-DBE quantitatively degraded to 2,4-dichlorophenoxyacetic acid in our large, continuous-flow laboratory ecosystems. No metabolite studies were performed on the individual batch cultures used for rate coefficient determinations. First-order transformation rate coefficients in the large laboratory ecosystems were determined from steady-state 2,4-DBE concentrations compared with input concentrations.

Depending on the experiment, second-order transformation rate coefficients were calculated by dividing k_1 by k_b, k_{AFDW}, or k_A. k_b was also determined with batch cultures of blended aufwuchs containing 50 mg

of candicidin per liter to eliminate nonbacterial microbial transformation. Candicidin, obtained from S.B. Penick & Co., was sterilized with ether and kept in a frozen aqueous suspension.

Large laboratory ecosystem tests. The suitability of the rate coefficients derived from batch cultures for predicting transformation rates in ecosystems was tested in our large laboratory aquatic ecosystem. The test consisted of four independent flowing channels (designated A, B, C, and D), each constituting a series of four 250-liter, continuously stirred tank reactors (CSTRs), designated 1 through 4. Water flowed successively through each set of CSTR microcosms. The CSTR conditions used for the 2,4-DBE study were the same as for earlier studies (2) except for the following changes. The water temperature was 15°C, and the channels were illuminated with 750 ft-c (8.07 klx) of fluorescent light in 8-h light–16-h dark cycles. These conditions discouraged the growth of filamentous green algae. Each CSTR was tested for 2,4-DBE transformation rates by introducing a continuous 2,4-DBE concentrate (to give a nominal concentration of 50 μg/liter in the CSTR), allowing the 2,4-DBE to decrease until a steady-state concentration was observed for several days, and then moving the 2,4-DBE concentrate to the next CSTR to undergo the same procedure. First-order transformation rate coefficients for these large flowing microcosms were calculated from steady-state 2,4-DBE concentrations compared with input concentrations.

Predicted k_1 values were calculated by multiplying the second-order rate coefficients by the corresponding parameter values determined for each CSTR. For example, k_b was used to predict k_1 in a CSTR by multiplying k_b by the bacterial concentration in the CSTR. CSTRs fluctuated in pH during the light-dark cycles, sometimes reaching a maximum pH of about 8.0. Under these conditions, we calculated that alkaline hydrolysis usually accounted for only a small percentage of 2,4-DBE transformation in the CSTRs. The k_1 values reported for the CSTRs are net microbial transformation rate coefficients with alkaline hydrolysis rate coefficients subtracted.

CSTRs A1 and B1 received a continuous input of low concentrations of nutrients (calculated ambient concentrations [milligrams per liter]: NO_3-N, 0.083; P, 0.010; C, 2.0). CSTRs A3 and B4 received a nutrient input nine times more concentrated than that for A1 and B1. These nutrient inputs were designed to yield large variations in microorganism concentrations in the suspended and aufwuchs phases among the microcosms.

2,4-DBE loss resulting from physical processes was negligible. Sorption and volatilization of 2,4-DBE in the CSTRs were tested in a channel with no nutrient inputs and therefore no significant microbial growth. No measurable loss occurred as a result of either of these processes. Sorption to microorganisms was tested with cultures of autoclaved aufwuchs. No measurable loss occurred as a result of sorption to the biomass.

RESULTS

Transformation rate coefficients based on total plate counts. Bacterial concentrations and k_1 and k_b values for each of 38 batch cultures containing microbiota from field sites and laboratory microcosms are shown in Table 1. Mean k_b values for blended aufwuchs were about one order of magnitude higher than those reported for suspended bacteria (3). However, the addition of candicidin, an antibiotic that rapidly kills nonbacterial cells (1), reduced the rate coefficients by about one order of magnitude. Therefore, the increased transformation rates were concluded to be a result of 2,4-DBE transformation by fungi and algae in the aufwuchs. This conclusion is supported by Paris et al. (3), who reported a similar k_b for one culture that contained visible algae.

To confirm that fungi were able to transform 2,4-DBE, we tested 11 genera of fungi, including 4 yeasts, in axenic cultures. Each culture except for three yeast genera rapidly transformed 2,4-DBE. Transformation in each case was stopped by the addition of candicidin. 2,4-DBE was transformed in axenic cultures of a green alga, *Chlamydomonas reinhardtii*, and a blue-green alga (cyanobacterium), *Anabaena flos-aquae*. Candicidin stopped 2,4-DBE transformation by the green but not by the blue-green alga.

Transformation rate coefficients based on biomass. CSTRs A1, A2, A3, B1, and B4 were chosen for periphyton biomass studies because their diverse nutrient inputs caused significant diversity in periphyton biomass. Total biomass (AFDW) concentrations ranged from 0.0046 g/liter in CSTR A2 to 0.072 g/liter in CSTR B4. The biomass concentrations used in the batch cultures ranged from 0.022 to 0.26 g/liter. First-order transformation rate coefficients were directly proportional to biomass concentrations in samples taken from the same CSTR, except for those taken from B4 (Table 2). The transformation rate per unit of biomass, however, varied among samples taken from different CSTRs, from 3.58 to 22.7 liters g^{-1} h^{-1}.

Transformation rate coefficients based on ratio of colonized surface area to container volume. For Teflon strips colonized with periphyton, transformation rates increased linearly as turbulence increased (Fig. 1), indicating a mass transport limitation. Transformation rates did not increase with increasing turbulence in batch cultures of blended periphyton. Mass transport limitation was also indicated in the large microcosms. As the paddlewheel speed in one of the CSTRs was increased from 2 to 12 rpm, the steady-state concentration of 2,4-DBE dropped from 8.4 ± 0.78 (standard deviation) to 2.6 ± 0.40 g/liter and then returned to 8.6 ± 0.72 g/liter when the speed was decreased back to 2 rpm. Using these observations as a guide, we constructed the k_A tester, a laboratory apparatus with a series of beakers stirred with paddles, for determining k_A at a known water velocity at the periphyton

TABLE 1. Rate coefficients for 2,4-DBE transformation in batch cultures of grab samples taken on various dates from microcosms and field sites

Sample source	CFU liter^{-1} (mean ± SD)[a]	k_1 h^{-1} ± SE	k_b (nl cell^{-1} h^{-1})[b]	
			Without candicidin	With candicidin[c]
CSTR				
A1	4.3 (±0.5) × 10^8	0.418 ± 0.018	0.97	
	4.9 (±0.4) × 10^8	0.366 ± 0.022	0.75	
A2	3.3 (±0.8) × 10^8	0.496 ± 0.038	1.5	
A3	1.2 (±0.1) × 10^9	0.930 ± 0.117	0.78	0.26
	8.3 (±0.8) × 10^8	0.609 ± 0.023	0.73	0.25
A4	2.3 (±0.2) × 10^9	0.932 ± 0.032	0.41	0.13
	1.7 (±0.1) × 10^9	0.517 ± 0.032	0.30	0.12
B1	6.6 (±0.8) × 10^8	0.346 ± 0.022	0.64	0.24
B3	1.5 (±0.04) × 10^7	0.204 ± 0.016	14	
	2.2 (±0.3) × 10^7	0.256 ± 0.012	12	
	5.0 (±0.7) × 10^8	0.730 ± 0.077	1.5	0.6
	5.0 (±0.9) × 10^8	0.813 ± 0.042	1.6	
B4	7.9 (±1.6) × 10^8	1.17 ± 0.084	0.15	
	5.2 (±0.7) × 10^8	0.995 ± 0.080	0.19	
Field site				
Harris Shoals	6.7 × 10^7	0.385 ± 0.025	5.7	
	2.2 × 10^8	0.462 ± 0.031	2.1	
	8.9 × 10^7	0.453 ± 0.013	5.1	0.05
Watson Mill	4.7 × 10^7	0.837 ± 0.026	18	0.12
	7.7 × 10^7	0.357 ± 0.007	4.6	
	1.4 × 10^8	0.783 ± 0.028	5.6	
	8.2 × 10^7	0.230 ± 0.009	2.8	
	1.7 × 10^8	0.604 ± 0.035	2.6	
	1.3 × 10^8	0.798 ± 0.041	6.1	
	2.3 × 10^8	0.847 ± 0.040	3.7	
Apalachee River	1.0 × 10^9	0.287 ± 0.040	0.29	0.052
	2.4 × 10^8	1.17 ± 0.070	4.9	0.11
Oconee River	8.5 × 10^8	1.75 ± 0.290	2.1	0.092

[a] Triplicate plates.
[b] The k_b for all CSTRs (mean ± standard deviation) was 2.5 ± 4.5 nl cell^{-1} h^{-1} (0.27 ± 0.17 nl cell^{-1} h^{-1} with candicidin); for the field sites it was 5.0 ± 4.3 and 0.085 ± 0.032 nl cell^{-1} h^{-1}, respectively.
[c] Used at 50 mg/liter in replicate cultures.

surface. The paddle rotation rates in the k_A tester were adjusted to approximate the water velocity in the large microcosm. With the k_A tester, k_1 was directly proportional to k_A, and k_A was constant (Fig. 2). For periphyton-colonized Teflon strips from the large microcosms and the municipal sewage treatment facility, the k_A was 23 ± 2.0 and 60 ± 3.5 liters m^{-2} h^{-1} (mean ± standard deviation), respectively.

TABLE 2. Rate coefficients based on biomass for 2,4-DBE transformation in batch cultures from large laboratory ecosystems

CSTR	AFDW (g/liter)	k_1 h^{-1}	k_{AFDW} (liters g^{-1} h^{-1})[a]
A1	0.034	0.42	12.4
	0.034	0.37	10.9
A2	0.022	0.41	18.6
	0.022	0.50	22.7
A3	0.130	0.61	4.69
	0.260	0.93	3.58
B1	0.025	0.35	14.0
	0.050	0.60	12.0
B4	0.120	1.17	9.75
	0.058	1.00	17.2

[a] Mean k_{AFDW} (± standard deviation) for all samples, 12.6 ± 5.9 liters g^{-1} h^{-1}.

Large laboratory ecosystem tests. The predicted k_1 values for each CSTR were based on the mean values for the three rate coefficients determined from batch cultures (Table 3). We predicted a k_1 range of 0.05 to 16 h^{-1} with k_b, a k_1 range of 0.06 to 0.91 h^{-1} with k_{AFDW}, and a relatively constant k_1 of 0.28 to 0.30 h^{-1} with k_A. The observed k_1 values among the CSTRs varied only by about a factor of 2, and the mean k_1 was 0.2 h^{-1}. Therefore, k_A most accurately predicted the observed 2,4-DBE transformation rates in our periphyton-dominated systems.

DISCUSSION

Transformation rate coefficients based on total plate counts or biomass apparently are not

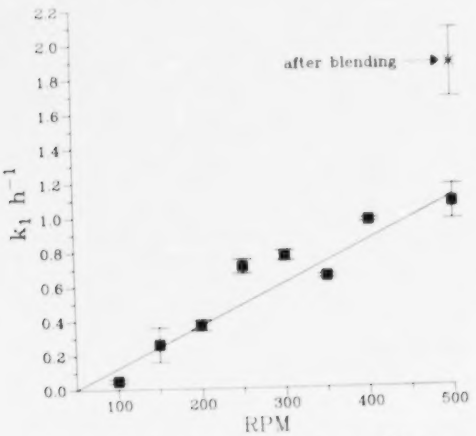

FIG. 1. Effect of mixing speed on 2,4-DBE transformation rates by periphyton attached to a Teflon strip. Mixing speeds were incrementally increased in the k_A tester. The maximum 2,4-DBE transformation rate was not reached with increases in speed. The maximum transformation rate (which should occur at the speed at which mass transport limitation is eliminated) was indicated by the transformation rate after the periphyton had been scraped and blended. Bars represent standard errors of regression of least-squares plots of the transformation rates.

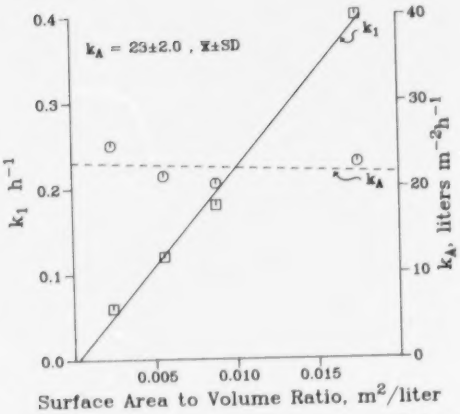

FIG. 2. 2,4-DBE transformation rate coefficients for various ratios of periphyton-colonized surface area to container volume. k_1 was directly proportional to this ratio, and k_A, calculated by dividing k_1 by the ratio, was constant.

suitable for periphyton communities, particularly when there is mass transport limitation. Because the transformation rates continued to increase even at high turbulence (Fig. 1), mass transport is probably a limiting factor for xenobiotic chemical transformations in most, if not all, periphyton-dominated systems. Therefore, transformation rate coefficients for periphyton-dominated systems should be indexed to a measurement of turbulence, such as the Reynolds number.

In previous experiments with methyl parathion and diethyl phthalate, we observed that total bacterial concentrations varied by orders of magnitude, but transformer population concentrations remained relatively constant, and therefore the transformation rates were consistent among diverse microcosms. This phenomenon is not surprising when the transformer population is smaller. When more nutrients are available, the organisms with the highest specific growth rates rapidly achieve numerical or mass dominance over those with lower specific growth rates. When only a small fraction of the bacteria are transformers and growth rates are randomly distributed over the whole population, then there is little chance that the transformers will have the highest growth rates and therefore greatly increase in concentration when available nutrient concentrations are increased. This phenomenon would affect to some degree the transformation rate for any chemical not transformed by all of the organisms in a mixed-organism environment. The magnitude of this effect would increase as the proportion of nontransformer organisms increased. We observed this phenomenon in mixed-batch cultures of suspended bacteria, in which the transformation rates of methyl parathion and diethyl phthalate were not

TABLE 3. Predicted and observed 2,4-DBE transformation rates in large laboratory ecosystems

CSTR	k_1 h^{-1} predicted by:			Observed k_1 h^{-1}
	k_b[a]	k_{AFDW}[b]	k_A[c]	
A1	0.48	0.11	0.28	0.12
A2	0.15	0.06	0.28	0.12
A3	2.1	0.89	0.28	0.26
A4	9.8		0.28	0.28
B1	0.83	0.16	0.28	0.21
B2	0.10		0.30	0.16
B3	0.05		0.30	0.22
B4	16.0	0.91	0.30	0.17

[a] Based on a mean k_b of 2.5 nl $cell^{-1}$ h^{-1} and total plate counts (suspended plus periphyton) for each CSTR.

[b] Based on a mean k_{AFDW} of 12.6 liters g^{-1} h^{-1} and AFDW concentrations for each CSTR.

[c] Based on ratio of colonized surface area to volume in the CSTRs, with a mean k_A of 23 liters m^{-2} h^{-1} (Fig. 2). k_A was determined by using Teflon strips colonized with periphyton in a low-nutrient CSTR during a later large-microcosm study independent of the 2,4-DBE experiments, in which the microcosms were cleaned and reinoculated with field microbiota.

significantly increased although the total bacterial concentrations increased by orders of magnitude after nutrient broth or other nutrients were added (2). Because of this phenomenon, we expected k_b to decrease as total bacterial concentration increased, as was observed for methyl parathion and diethyl phthalate with aufwuchs (2). The decreasing k_b resulted from increases in the proportion of nontransformers.

We hoped to avoid varying the ratio of transformers to nontransformers by using 2,4-DBE. This compound was rapidly transformed by virtually all the bacteria we isolated on nutrient agar plates; therefore, we expected transformation rates to be proportional to total plate counts in microcosms. However, k_1 was consistent among CSTRs containing different bacterial concentrations. Because of the mass transport limitation, periphyton contact with 2,4-DBE was probably restricted to those microorganisms near the surface. Even with this complication, however, the concentration of bacteria near the periphyton surfaces should have varied considerably between CSTRs with different nutrient environments. Therefore, we still expected k_1 in the CSTRs to be somewhat proportional to total plate counts. The problem with normalizing 2,4-DBE transformation rates to plate counts in our laboratory ecosystems resulted from neglecting the rapid transformation activity of other classes of microorganisms in the procedure. When bacterial concentrations were low, the difference in transformation activity apparently was made up by increased activity by fungi or other microorganisms.

The rate coefficient k_A was constant over an order-of-magnitude range in the ratio of surface area to volume (Fig. 2) and worked well for predicting 2,4-DBE transformation rates in our large laboratory ecosystems. The constancy of k_1 for methyl parathion and diethyl phthalate transformation in large laboratory ecosystems with equal surface area/volume ratios in our earlier experiments (2) supports the applicability of k_A to periphyton-dominated ecosystems. It is a convenient, non-site-specific coefficient that requires only an estimate of the submerged surface area, water volume, and flow rate (turbulence) of an aquatic system.

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LITERATURE CITED

1. **Lampen, J. O.** 1969. Amphotericin B and other polyenic antifungal antibiotics. Am. J. Clin. Pathol. **52**:138–146.
2. **Lewis, D. L., and H. W. Holm.** 1981. Rates of transformation of methyl parathion and diethyl phthalate by aufwuchs microorganisms. Appl. Environ. Microbiol. **42**:698–703.
3. **Paris, D. F., W. C. Steen, G. L. Baughman, and J. T. Barnett, Jr.** 1981. Second-order model to predict microbial degradation of organic compounds in natural waters. Appl. Environ. Microbiol. **41**:603–609.
4. **White, A., P. Handler, and E. L. Smith.** 1964. Principles of biochemistry. McGraw-Hill Publications, New York.

ERRATUM

Predicting 2,4-Dichlorophenoxyacetic Acid Ester Transformation Rates in Periphyton-Dominated Ecosystems

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Volume 46, no. 1, p. 149, column 2, lines 1 and 2: "... $\underline{k}_1$ was directly proportional to $k_{\underline{A}}$, and $\underline{k}_{\underline{A}}$ was constant" should read "... $\underline{k}_1$ was directly proportional to the ratio of colonized surface area to container volume, and $\underline{k}_{\underline{A}}$ was constant."